Respiration in Bruised Fruit Tissue. A Preliminary Report*

By R. L. Pollack, G. D. Chase and J. L. Rabinowitz

From the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, Philadelphia College of Pharmacy and Science and Radioisotope Service, Veterans Administration Hospital, Philadelphia, Pennsylvania

Introduction

A series of studies performed in our laboratories has been concerned with the respiratory activity of the red tart cherry (Prunus cerasus) during normal growth [1] and the changes in respiratory activity upon bruising [2, 3]. It was shown that the respiratory output of carbon dioxide increases markedly upon bruising the fruit, without an equivalent increase in the amount of oxygen consumed. ^{14}C-labeled metabolic substrates were then used to investigate further this increase in carbon dioxide output which results from bruising. Wang et al. [5] showed that ^{14}C-labeled carbon appeared as part of the respiratory carbon dioxide output following the injection of acetate-1-^{14}C.

The present study is an exploratory investigation of the carbon dioxide output (both total and labeled) resulting from the normal and bruised metabolic activity following the administration of various labeled substrates.

Materials and Methods

Solution Used

No.	Compound	Concentration	Specific Activity
1	Acetate-1-^{14}C	48.8×10^{-4} μmole/μl 97.6×10^{-4} μC/μl	2.00 μC/μmole
2	Acetate-2-^{14}C	48.8×10^{-4} μmole/μl 60.0×10^{-4} μC/μl	1.23 μC/μmole
3	Citrate-1,5-^{14}C	48.8×10^{-4} μmole/μl 48.8×10^{-4} μC/μl	1.00 μC/μmole

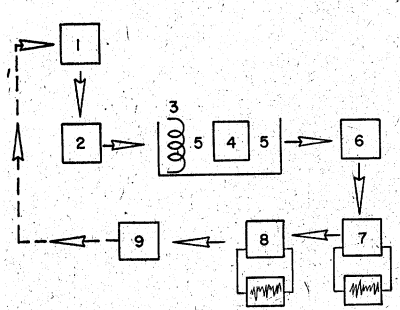


Figure 1. Diagrammatic representation of sealed CO_2 detection system
1. Low-velocity air pumps
2. Humidying chamber (acidified water)
3. Quarter-inch copper tubing coil to warm air prior to entering fruit chamber
4. Fruit chamber
5. Constant-temperature water bath
6. Drying tube
7. Cary Model 31 Vibrating-Reed electrometer and Sargent Model MR recorder
8. Beckman Model IR-4 infrared spectrophotomer
9. Flowmeter

The fruit used in the experiments was obtained from the orchard of the Delaware Valley College of Science and Agriculture. Cherries were picked from the same trees that had supplied the fruit for all previous studies and were handled as reported [1, 3].

The physical system used to determine both the total and labeled CO_2 is a modification of that used by Tolbert, Kirk and Baker [4]. The present method differs in that the system is a closed one, i. e. the CO_2 produced is recirculated rather than being removed (Figure 1). The volume of the enclosed atmosphere in the system is sufficiently great that the oxygen consumed and CO_2 released produce a negligible change in composition of the enclosed atmosphere.

Total carbon dioxide was determined by means of infrared spectroscopy using the Beckman IR-4. Ten centimeter gas cells were used for both sample and reference. The sample cell had both inflow and outflow orifices connected to the remainder of the system with ball and socket joints. The reference cell was closed at the same time as the sample system. The wave length drive instrument was adjusted for maximum absorption to carbon dioxide.

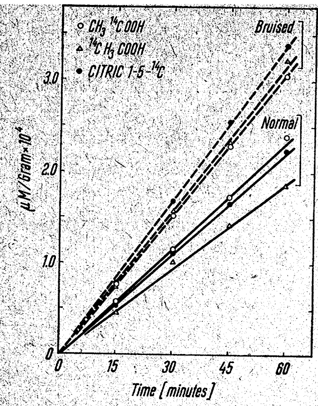
$^{14}CO_2$ was determined by a Cary Model 31 Vibrating Reed electrometer.

Two series of experiments were conducted. The first involved the injection of a labeled substrate into control fruits (carefully protected from being bruised) followed by the simultaneous determination of both the total and ^{14}C-labeled fraction of the respiratory CO_2 output.

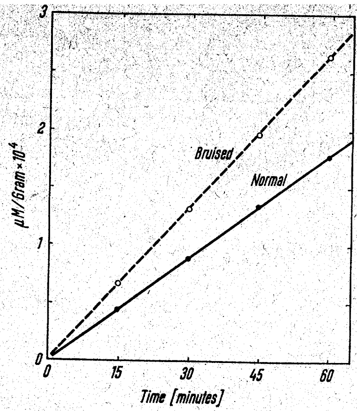
The second series was an exact duplicate of the first except that the fruit was bruised following the injection of the labeled substrate. Again measurements were made of the total and labeled CO_2 output.

Each determination was made on a lot consisting of six cherries. The fruit in each lot was weighed and then injected with the prepared labeled solutions on the basis of 1 μl of solution per gram of fresh weight. Injection was carried out by inserting a 27 gauge needle through the bit of remaining stem and into the flesh beside the pit. The syringe was held between the thumb and fingers so the syringe could be rolled and the needle gradually worked into the stem; pushing the needle directly into

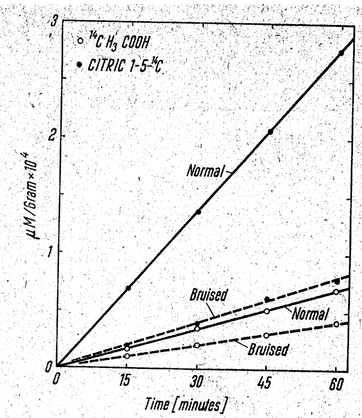
* Presented in part at the VI International Congress of Biochemistry, New York, N. Y., July 1964



2



3



4

Figure 2. Rate of total CO_2 production from normal and bruised fruit following the injection of ^{14}C-labeled substrates (solutions 1,2 and 3) — **Figure 3.** Rate of $^{14}CO_2$ production from normal and bruised fruit following the injection of acetate-1-^{14}C (solution 1) — **Figure 4.** Rate of $^{14}CO_2$ production from normal and bruised fruit following the injection of acetate-2-^{14}C (solution 2) and citrate-1,5-^{14}C (solution 3)

the stem would cause the stem area to be pushed into the fruit with the possibility of subsequent bruising. Upon withdrawal of the needle (again by rolling it out) the stem end was sealed with collodion.

Following the injection of a substrate, the fruit of each lot was individually deposited into sample chamber. The chamber was then connected into the closed system and lowered into the water bath which was maintained at 30° C.

Table 1. Amounts of total CO_2 and $^{14}CO_2$ obtained from bruised and unbruised cherries pre-injected with ^{14}C-labeled substrates

Time [min]	Total CO_2 [μmole/g]		$^{14}CO_2$ [μmole/g $\times 10^4$]		$^{14}CO_2$ [mole % $\times 10^3$]	
	Controls	Bruised	Controls	Bruised	Controls	Bruised
			$CH_3\,^{14}COOH$			
15	0.57	0.77	0.453	~~0.066~~	7.95*	8.58
30	1.15	1.52	0.89	~~0.132~~	7.73	8.68
45	1.73	2.29	1.35	~~0.197~~	7.80	8.61
60	2.39	3.05	1.79	~~0.264~~	7.50	8.65
					7.74	8.63
			$^{14}CH_3\,COOH$			
15	0.46	0.75	0.174	0.100	3.78	1.33
30	0.92	1.59	0.369	0.212	4.02	1.33
45	1.42	2.34	0.518	0.311	3.65	1.33
60	1.85	3.22	0.702	0.411	3.79	1.28
					3.81	1.32
			Citric Acid-1,5-^{14}C			
15	0.54	0.77	0.69	0.195	12.8	2.53
30	1.11	1.68	1.37	0.389	12.3	2.32
45	1.66	2.55	2.07	0.621	12.5	2.42
60	2.24	3.38	2.76	0.783	12.3	2.32
					12.5	2.40

* $\frac{0.453 \times 10^{-4}\ \mu\text{mole}}{0.57\ \mu\text{mole}} \times 100 = 7.95 \times 10^{-3}$ mole % $^{14}CO_2$ (atom % ^{14}C)

Bruising was accomplished by firmly rolling each fruit between the concave surfaces of two watch glasses, care being taken not to break the skin at any point on the surface of the fruit. The fruit was bruised just prior to being placed into the chamber.

The system was sealed and air circulation started. Monitoring of both the total and labeled carbon dioxide was started immediately, but the system was allowed to equilibrate for at least one-half hour before data were recorded for use.

Results

The average results obtained for each precursor are recorded in Table 1. Information is presented for 15 minute periods up to an hour in order to show the constancy of output for each period.

The carbon dioxide output for both the normal and bruised lots of fruits, following the injection of the three labeled substrates, is shown in Figure 2. Following the same pattern of activity shown in previous studies [2, 3] the total CO_2 output increased after bruising and ranged from 130% to 171% of the normal value (Table 2).

An increase in $^{14}CO_2$ output following bruising, is also observed when carboxy-labeled acetate is injected (Figure 3), averaging 142% of normal. With the use of methyl-labeled acetate and citrate-1,5-^{14}C, the results are the reverse (Figure 4); the output of $^{14}CO_2$ following bruising decreased from 57.4% of normal in the case of 2-^{14}C-acetate to 29.1% of normal with citrate.

Discussion

The increase in total carbon dioxide, following bruising, could be due to the increase of decarboxylation as a result of the liberation of decarboxylases from the injured cells. These decarboxylases act not only upon the substrate pool present in the intra-cellular space but on those present in the extra-cellular spaces as well.

Table 1. Amounts of total CO_2 and $^{14}CO_2$ obtained from bruised and unbruised cherries pre-injected with ^{14}C-labeled substrates.

Time (min)	Total CO_2 (µmole/g)		$^{14}CO_2$ (µmole/g x 10^4)		$^{14}CO_2$ (mole % x 10^3)	
	Controls	Bruised	Controls	Bruised	Controls	Bruised
			$CH_3\ ^{14}COOH$			
15	0.57	0.77	0.453	0.66	7.95*	8.58
30	1.15	1.52	0.89	1.32	7.73	8.68
45	1.73	2.29	1.35	1.97	7.80	8.61
60	2.39	3.05	1.79	2.64	7.50	8.65
					7.74	8.63
			$^{14}CH_3\ COOH$			
15	0.46	0.75	0.174	0.100	3.78	1.33
30	0.92	1.59	0.369	0.212	4.02	1.33
45	1.42	2.34	0.518	0.311	3.65	1.33
60	1.85	3.22	0.702	0.411	3.79	1.28
					3.81	1.32
			Citric Acid-1,5-^{14}C			
15	0.54	0.77	0.69	0.195	12.8	2.53
30	1.11	1.68	1.37	0.389	12.3	2.32
45	1.66	2.55	2.07	0.621	12.5	2.42
60	2.24	3.38	2.76	0.783	12.3	2.32
					12.5	2.40

* $\dfrac{0.453 \times 10^{-4}\ \mu mole}{0.57\ \mu mole} \times 100 = 7.95 \times 10^{-3}$ mole % $^{14}CO_2$ (atom % ^{14}C)

Table 2. Data summary

	$CH_3\,^{14}COOH$	$^{14}CH_3\,COOH$	Citric Acid —1,5 — ^{14}C
Total CO_2 (μmole/g/h)			
Normal	2.34	1.86	2.20
Bruised	3.05	3.18	3.36
% of Normal	130	171	153
Dose injected (μmole/g $\times 10^4$)			
Normal	55.0	58.5	65.1
Bruised	57.0	59.7	63.1
$^{14}CO_2$ (μmole/g/h $\times 10^4$)			
Normal	1.79	0.70	2.76
Bruised	2.64	0.41	0.78
$^{14}CO_2$ (% of dose/h)			
Normal	3.25	1.195	4.24
Bruised	4.63	0.687	1.236
% of Normal	142.6	57.4	29.1
Number of runs averaged in above tabulations	2	2	3

By use of specifically-labeled compounds it has been demonstrated that the effect of bruising was not totally confined to an over-all increase in the amount of carbon dioxide involved. The use of labeled citrate dramatically showed that although the total CO_2 increases with bruising, the portion of CO_2 directly attributable to the citrate ($^{14}CO_2$) decreased following bruising, rather than following the over-all trend. It is suggested that such a marked decrease in the liberation of $^{14}CO_2$ may be accounted for by assuming that the utilization of citrate follows a different pathway following bruising, and in some manner, the products of citrate utilization are shunted aside from the normal pathway, and do not follow the same sequence of metabolic events as does, for example, carboxy-labeled acetate.

Acknowledgment

The authors wish to thank Professor J. Feldstein of the Delaware Valley College of Science and Agriculture for his cooperation during this study.

References

[1] Pollack R. L., Hoban N. and Hills C. H.: Proc. Am. Soc. Hort Sci. *78*, 86 (1961)
[2] Pollack R. L., Ricciuti C., Woodward C. F. and Hills C. H.: Food Technol *12*, 102 (1958)
[3] Pollack R. L., Whittenberger R. T. and Hills C. H.: Food Technol. *12*, 106 (1958)
[4] Tolbert B. M., Kirk M. and Baker E. M.: Am. J. Physiol. *185*, 269 (1956)
[5] Wang C. H., Hansen E. and Christensen B. E.: Plant. Physiol. *28*, 741 (1953)

Authors' addresses: R. L. Pollack, Ph. D., Assoc. Prof., Department of Biochemistry, Schools of Dentistry and Pharmacy, Temple University, Phila., Pa.; G. D. Chase, Ph. D., Prof., School of Chemistry, Philadelphia College of Pharmacy and Science, Phila., Pa.; J. L. Rabinowitz, Ph. D., Chief, Radioisotope Service Veterans Administration Hospital, Phila., Pa., 19104.

Respiration in Bruised Fruit Tissue

1. A closed system was developed for the simultaneous measurement of total CO_2 and $^{14}CO_2$ obtained from a small group of fruit preinjected with C^{14}-labeled substrates. — 2. The total carbon dioxide output of bruised fruit was observed to be larger than for normal fruit, following the administration of 1-^{14}C-acetate, 2-^{14}C-acetate, and 1,5-^{14}C-citrate. — 3. Following the administration of 1-^{14}C-acetate, the $^{14}CO_2$ output was observed to be higher for the bruised than for the normal fruit, while for 2-^{14}C-acetate and 1,5-^{14}C-citrate, the results were reversed. — 4. The results obtained for the total carbon dioxide output may be explained by assuming an over-all increase in the metabolic activity brought about by bruising. The decrease noted in the output of $^{14}CO_2$ after the use of 2-^{14}C-acetate and the 1,5-^{14}C-citrate may come about, during the increased activity, as a result of their being depleted through utilization via a different metabolic pathway initiated by the bruising phenomenon.

Über die Atmung in gequetschten Fruchtgeweben

1. Für die gleichzeitige Messung von Gesamt-CO_2 und $^{14}CO_2$, das von einer kleinen Menge an Früchten, die mit ^{14}C-markierten Substanzen vorbehandelt waren, entwickelt wird, wurde ein geschlossenes System entwickelt. — 2. Nach Anwendung von Acetat-1-^{14}C, Acetat-2-^{14}C und Citrat-1,5-^{14}C zeigte sich bei gequetschten Früchten eine größere Gesamt-CO_2-Entwicklung als bei normalen Früchten. — 3. Nach Anwendung von Acetat-1-^{14}C zeigten gequetschte Früchte eine höhere $^{14}CO_2$-Entwicklung als normale, während Acetat-2-^{14}C und Citrat-1,5-^{14}C umgekehrte Ergebnisse erbrachten. — 4. Die Ergebnisse bezüglich der Gesamt-CO_2-Entwicklung können auf eine allgemeine Steigerung des Stoffwechsels durch die Quetschung zurückgeführt werden. Das bei Anwendung von Acetat-2-^{14}C und Citrat-1,5-^{14}C beobachtete Absinken der $^{14}CO_2$-Entwicklung während der allgemein gesteigerten Stoffwechselaktivität könnte angesehen werden als Folge einer Umsetzung über einen anderen Stoffwechselweg, der durch das Quetschen der Früchte verursacht wurde.

La respiration des tissus de fruits écrasés

1. Un système complet a été mis au point assurant la mesure simultanée de CO_2 et de $^{14}CO_2$ obtenu à partir d'une faible quantité de fruits préparés avec des substances marquées au ^{14}C. — 2. Après application d'acétate-1-^{14}C, d'acétate-2-^{14}C et de citrate-1,5-^{14}C, les fruits écrasés développent une plus grande quantité de CO_2 que les fruits normaux. — 3. Après application d'acétate-1-^{14}C, les fruits écrasés développent une plus grande quantité de $^{14}CO_2$ que les fruits normaux, alors que l'acétate-2-^{14}C et le citrate-1,5-^{14}C donnent des résultats inverses. — 4. Les résultats concernant le développement de CO_2 peuvent reposer sur une intensification générale du métabolisme par l'effet de l'écrasement. La diminution du développement de $^{14}CO_2$ observable dans le cas de l'application d'acétate-2-^{14}C et de citrate-1,5-^{14}C, alors que le métabolisme général s'élève, pourrait être considérée comme la conséquence d'une modification d'itinéraire entraînée par l'écrasement des fruits.